FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT' ENTERED AT 13:37:05 ON 10 MAY 2000

L1	1873	S	KAWASHIMA E?/AU OR FARINELLI L?/AU OR MAYER P?/AU
L2	35	S	SEQUENCING AND L1
L3	2	S	PRIMER AND L2
L4	864	S	MULTIPLE TARGET
L5	16	S	L4 AND SEQUENCING
L6	1	S	L5 AND PRIMER EXTENSION
L7	287	S	REPETITIVE SEQUENCES AND PRIMER EXTENSION
L8	42	S	L7 AND SEQUENCING
L9	0	S	LABEL AND L8
L10	9	S	POLYMERASE AND L8
L11	668	S	SAME SEQUENCES
L12	0	S	L8 AND L11

WEST

Generate Collection

Search Results - Record(s) 1 through 10 of 10 returned.

☐ 1. Document ID: US 6027890 A

L11: Entry 1 of 10 File: USPT Feb 22, 2000

US-PAT-NO: 6027890
DOCUMENT-IDENTIFIER: US 6027890 A
TITLE: Methods and compositions for enhancing sensitivity in the analysis of biological-based assays

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6027889 A

L11: Entry 2 of 10 File: USPT Feb 22, 2000

US-PAT-NO: 6027889
DOCUMENT-IDENTIFIER: US 6027889 A
TITLE: Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6013431 A

L11: Entry 3 of 10 File: USPT Jan 11, 2000

US-PAT-NO: 6013431
DOCUMENT-IDENTIFIER: US 6013431 A
TITLE: Method for determining specific nucleotide variations by primer extension in the presence of mixture of labeled nucleotides and terminators

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 4. Document ID: US 5935793 A

L11: Entry 4 of 10 File: USPT Aug 10, 1999

US-PAT-NO: 5935793
DOCUMENT-IDENTIFIER: US 5935793 A
TITLE: Parallel polynucleotide sequencing method using tagged primers

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 5. Document ID: US 5912129 A

L11: Entry 5 of 10 File: USPT Jun 15, 1999

US-PAT-NO: 5912129
DOCUMENT-IDENTIFIER: US 5912129 A
TITLE: Multi-zone polymerase/ligase chain reaction

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 6. Document ID: US 5709997 A

L11: Entry 6 of 10 File: USPT Jan 20, 1998

US-PAT-NO: 5709997
DOCUMENT-IDENTIFIER: US 5709997 A
TITLE: Nucleic acid detection of hepatitis GB virus

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 7. Document ID: US 5645801 A

L11: Entry 7 of 10 File: USPT Jul 8, 1997

US-PAT-NO: 5645801
DOCUMENT-IDENTIFIER: US 5645801 A
TITLE: Device and method for amplifying and detecting target nucleic acids

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 8. Document ID: US 5525463 A

L11: Entry 8 of 10 File: USPT Jun 11, 1996

US-PAT-NO: 5525463
DOCUMENT-IDENTIFIER: US 5525463 A
TITLE: Method and reagents for detection of mycobacteria using superoxide dismutase gene targeting

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 9. Document ID: US 5518900 A

L11: Entry 9 of 10 File: USPT May 21, 1996

US-PAT-NO: 5518900
DOCUMENT-IDENTIFIER: US 5518900 A
TITLE: Method for generating single-stranded DNA molecules

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 10. Document ID: US 5415839 A

L11: Entry 10 of 10 File: USPT May 16, 1995

US-PAT-NO: 5415839
DOCUMENT-IDENTIFIER: US 5415839 A
TITLE: Apparatus and method for amplifying and detecting target nucleic acids

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

Generate Collection

Term	Documents
(9 AND 10).USPT,DWPI.	10

Display 50 Documents, starting with Document: 10

Display Format: TI Change Format

WEST

Help | Logout | Interrupt

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers | Preferences

Search Results -

Term	Documents
(9 AND 10).USPT,DWPI.	10

Database: US Patents Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: 19 and 110 Clear

Search History

Today's Date: 5/10/2000

DB Name	Query	Hit Count	Set Name
USPT,DWPI	19 and 110	10	L11
USPT,DWPI	plurality same nucleic acid molecules or plurality same target	17218	L10
USPT,DWPI	18 and polymerase	81	L9
USPT,DWPI	17 and primer extension	83	L8
USPT,DWPI	sequencing and 16	196	L7
USPT,DWPI	immobilized same primers	297	L6
USPT,DWPI	immobilzed same primers	0	L5
USPT,DWPI	12 and primer	2	L4
USPT,DWPI	11 and primer extension	1	L3
USPT,DWPI	sequencing and 11	5	L2
USPT,DWPI	Kawashima-e$.in. or farinelli-l$.in. or mayer-p$.in.	132	L1

WEST

Generate Collection

L2: Entry 4 of 5 File: DWPI Jan 19, 2000

DERWENT-ACC-NO: 1998-568282
DERWENT-WEEK: 200009

TITLE: New nucleic acid amplification - by extension of immobilised primers annealed to target, separation of strands, annealing extended primer to second primer and repeating extension

ABTX:< br>USE - The method is used to produce NA for sequencing, diagnosis and screening, as supports for other materials, for generating free NA (particularly in situ RNA synthesis), monitoring gene expression, identifying NA encoding rarely expressed gene products and identifying heterozygotes and in NA fingerprinting.

INNM:
FARINELLI, L

INNM:
KAWASHIMA, E

INNM:
MAYER, P

WEST

Generate Collection

L2: Entry 5 of 5 File: DWPI Jan 19, 2000

DERWENT-ACC-NO: 1998-557136
DERWENT-WEEK: 200009

TITLE: Method for sequencing nucleic acid molecules - comprises use of single stranded nucleic acid molecules and labelled nucleotides

ABTX:
Novel method for sequencing nucleic acid molecules (NAMs), comprises: (a) providing at a first location a number of single stranded (ss) NAMs that have the same sequences as one another and that are hybridised to primers in a manner to allow primer extension in the presence of nucleotides and a nucleic acid polymerase (NAP); (b) providing at a second location, which is different from the first location, a number of ss NAMs that have the same sequences as one another, but that have different sequences from the ss NAMs at the first location, and that are also hybridised to primers in a manner to allow primer extension in the presence of nucleotides and a NAP; (c) providing each location with NAP and a given labelled nucleotide under conditions that allow extension of the primers if a complementary base or if a number of such bases are present at the appropriate position in the ss NAMs; (d) detecting whether or not the labelled nucleotide has been used for primer extension at each location by determining whether or not the label present on the nucleotide has been incorporated into extended primers, and (e) repeating steps (c) and (d) one or more times so that the extended primers comprising a number of labels are provided. Also claimed is an apparatus for performing the method, comprising a number of nucleotides, NAP and a detection means for performing step (d), the detection means being adapted to distinguish between the different locations

INNM:
FARINELLI, L

INNM:
KAWASHIMA, E H

INNM:
MAYER, P

INNM:
KAWASHIMA, E

WEST

Generate Collection

L5: Entry 2 of 5 File: USPT Aug 10, 1999

DOCUMENT-IDENTIFIER: US 5935793 A
TITLE: Parallel polynucleotide sequencing method using tagged primers

ABPL:
The invention is directed to a method for sequencing multiple target polynucleotide segments in parallel, and to compositions and kits therefor. In the method, a plurality of sample polynucleotide fragments are used to form a mixture of different-length sequencing fragments. The sequencing fragments are complementary to at least two different sample fragments, wherein (1) each sequencing fragment terminates at a predefined end with a known base or bases, and (2) each sequencing fragment contains an identifier tag sequence that identifies the sample fragment to which the sequencing fragment corresponds. The sequencing fragments are then separated on the basis of size to produce a plurality of resolved, size-separated bands. Resolved bands are collected in separate aliquots, which, in a preferred embodiment, are then subjected to an amplification step to amplify the complements of the tag sequences in each aliquot, and preferably, the tag sequences too. Amplification is preferably by PCR. The (amplified) aliquots are then separately hybridized with an array of immobilized different-sequence tag probes under conditions effective to provide specific hybridization of tag sequences, or of tag sequence complements, with the corresponding immobilized tag probes, to form a hybridization pattern on the array, from which sequence information of one or more sample fragments are determined.

BSPR:
With respect to the above tag-primer embodiment, it is also advantageous to use a plurality of different-sequence cloning vectors to enable the simultaneous creation of sequencing fragments from a plurality of different sample templates (also referred to as a template pool) in a single extension reaction mixture. Thus, in this embodiment, step (1) above is performed on a plurality of separate, different-sequence tag-vectors, each different-sequence tag-vector having (i) a cloning site, (ii) located on the 3'-side of the cloning site, a first vector primer sequence which contains a vector-identifier tag region which is unique for each different-sequence tag-vector, to form a plurality of libraries of different-sequence tag-vectors, step (2) is modified to include isolating at least one clone from each different-sequence tag-vector clone library, and step (3) includes mixing together a clone isolated from each different-sequence tag-vector library before said hybridizing, to form a clone mixture. By this approach, a single tag-primer can be used to generate sequencing fragments from a plurality of different sample fragments in a single primer-extension reaction mixture, thus streamlining template preparation and reducing the number of primer extension reactions. Each sequencing fragment product in the extension reaction mixture contains a tag sequence from the extension tag-primer that identifies the pool of tag-vectors from which the fragment was generated and optionally, the terminating base type(s) of the fragments. Each sequencing fragment product also contains a vector-identifier tag sequence which identifies the vector in which the source sample sequence was cloned. The combination of vector tag and primer tag uniquely identifies the sample fragment to which each sequencing fragment corresponds.

BSPR:
In a second general embodiment, the method of the invention involves the use

of a plurality of separate, different-sequence vectors, referred to herein as tag-vectors, each containing a unique identifier tag. Each different-sequence tag-vector includes (i) a cloning site, (ii) located to the 3'-side of the cloning site, an identifier tag which is unique for each different-sequence tag-vector, and (iii) located on the 3'-side of the identifier tag, a first primer region. In practicing this embodiment, polynucleotide sample fragments are inserted or cloned into a plurality of each separate, different-sequence tag-vector, to form a plurality of separate libraries of tag-vector clones. Individual clones are selected from each of at least two such libraries and are combined. The combined clones may then be used to form a sequencing fragment mixture by primer extension, for size-fractionation and sequencing analysis as above.

DEPR:
As discussed below, different-sequence sample fragments are combined with unique-sequence identifier tags to allow tracking and identification of the sample fragments for sequence determination. In one embodiment, the tags are linked to polymerization primers which are used to generate sequencing fragments via primer extension reactions. In other embodiments, tags are included in cloning vectors which are used to link unique tags to different sequencing fragments.

DEPR:
FIG. 1A shows an exemplary tag-primer 20 containing a unique tag sequence 22, an optional linker region 24, and a primer sequence 26 located to the 3'-side of tag sequence 22 and optional linker region 24. Primer sequence 26 is preferably a "universal" primer sequence for initiating polymerase-mediated primer extension on a conventional cloning vector. Tag sequence 22 may be linked directly to the primer sequence via a phosphorus internucleotide linkage, or via linker region 24 which may be a polynucleotide or non-polynucleotide linker. Primer sequence 26 is also useful as a primer template for preparing multiple copies of the sequence complement of regions 22, 24, and 26 by linear amplification, as discussed further below.

DEPR:
FIG. 1B shows a tag-primer 40 (primer-tag-primer) which includes identifier tag sequence 42, a primer sequence 44 located on the 3'-side of identifier tag sequence 42, and a second primer sequence 46 located on the 5'-side of tag sequence 42. Primer sequences 44 and 46 may be spaced from tag sequence 42 by intervening linkers (not shown). In addition to having the features noted with respect to tag-primer 20 above, tag-primer 40 is amenable to PCR (polymerase chain reaction) amplification of the segment spanning sequences 42, 44 and 46 using repeated cycles of primer binding and primer extension using corresponding third and fourth primers to amplify the identifier tag sequences and their complements. In other words, one of the third and fourth primers contains a sequence complementary to primer sequence 44, and the other contains substantially the same sequence as primer sequence 46 or a portion thereof. An important advantage of primer-tag-primers of the type shown in FIG. 1B is that they allow rapid exponential amplification of the tag identifier in each sequencing fragment without amplifying the sample fragment sequences. This results in an increased quantity of identifier tag with a relative reduction in sample-derived background, so that sensitivity for detecting the identifier tag on a probe-array can be substantially increased.

DEPR:
FIG. 2B shows a tag vector 60 which includes cloning site 70; a tag sequence 62 located on the 3'-side of the cloning site; and a primer sequence 66 located on the 3'-side of the identifier tag. Cloning site 70 preferably occurs only once in the vector, for inserting a sample fragment into the vector by ligation. Hybridization of an initiating primer to primer template sequence 66, followed by primer extension, affords sequencing fragments which are complementary to the vector template, each containing sequence complements of the primer template sequence and the identifier tag sequence from the vector. As discussed below, this type of tag vector does not require the use of tag primers.

DEPR:
In a first embodiment for use in preparing sequencing fragments, wherein tag-primers (e.g., FIGS. 1A-1B) and a single cloning vector are employed, the sample fragments are inserted into a plurality of identical cloning vectors by standard ligation techniques, to form a mixture of sequencing vectors each containing a different sample fragment. The mixture of sequencing vectors is plated or otherwise dispersed on a growth-promoting substrate, typically an agar-based solid medium, under dilute conditions such that individual homogeneous clones can be isolated, each containing a different sequencing vector. Typically, a plurality of individual clones (usually still contained in host cells) are removed from the substrate and are each transferred to separate vessels containing a suitable growth medium, to increase the amount of DNA (or RNA) available for sequencing. The sequencing vectors are then isolated from each vessel and kept separate from each other for subsequent use as primer-extension templates.

DEPR:
Sequencing fragments may be generated from each sequencing vector template using any of a number of approaches, depending in part on whether more than one label type is being used for detection. Assuming that only a single label is to be used, each sequencing vector template is divided into four separate aliquots, one for each possible terminating base-type, for conducting primer extension reactions.

DEPR:
In one embodiment, each of the four aliquots for a given vector template is reacted with a different tag-primer, and primer extension is carried out using a DNA polymerase in the presence of four deoxynucleotide triphosphates (dNTPs), with a different dideoxy terminator for each aliquot if the Sanger approach is used. Each reaction mixture produces a ladder of sequencing fragments all terminating with the same base-type, and each having the same identifier tag to indicate both the particular sample fragment and the terminator base type for the sequencing fragments produced in that reaction. Thus, for each different sequencing vector template, the product sequencing fragments contain a total of four different identifier tags for that template.

DEPR:
If the sample fragments are provided as a plurality of different vector libraries prior to hybridization, as discussed with reference to FIG. 2A above, a clone from each library can be mixed together to form a clone mixture (also referred to as a template pool) in which each different vector clone is uniquely identified by its vector-identifier tag sequence (54 in FIG. 2A). The clone mixture can be divided into four aliquots as above for primer extension reactions. Each of the four aliquots is reacted with a plurality of tagged primers that all include (i) a first tag region that is identical among all the primers used in the aliquot, for identifying the terminating base-type of the aliquot reaction mixture, and (ii) a second, vector-tag identifier region for hybridizing to the corresponding vector-identifier tag region in each different vector clone in the aliquot to initiate primer extension. A plurality of such template pools can be prepared from the libraries and can be loaded into separate vessels (up to four vessels per template pool for the four terminator base types) for performing multiple chain extension reactions in parallel. The reaction mixtures may then be mixed together for separation on the basis of fragment length. Each sequencing fragment carries a tag sequence that identifies the source template pool, the particular vector type, and terminator base-type.

DEPR:
In a second general embodiment for preparing sequencing fragments, tag-vectors are employed, such as illustrated in FIG. 2B. The sample fragments are inserted into a plurality of separate, different-sequence tag-vectors to form separate libraries of tag-vector clones. Each library contains vectors all having the same identifier tag but different sample fragment inserts. Each

library is then separately plated or otherwise dispersed to produce individually isolable clones. A clone is selected from each of at least two of the plated libraries, and the selected clones are combined and are (optionally) grown together in a growth medium for a selected time, or until a selected density has been obtained, to amplify the amount of clonal material for sequencing. The mixture of sequencing vectors is then isolated from the growth medium for use as primer extension template.

DEPR:
Sequencing fragments may be generated from the sequencing vector mixture using a single universal primer which is effective to initiate primer extension through the sample fragment inserts in the vectors. The primer extension reactions may be conducted together using a single aliquot of the vector mixture if four different labels attached to the 3'-terminator bases are used to distinguish the terminating base-types. Alternatively, when a four-label method is used wherein the labels are carried on the extension primer, the primer extension reactions may be separately conducted in four different aliquots, one for each base-type, which upon completion may be combined for all subsequent processing steps.

DEPR:
It should be noted that when tag-vectors are used in accordance with the second embodiment, primer extension beyond the identifier tag regions of the templates leads to incorporation of tag sequence complement regions near 5'-end regions of the nascent sequencing fragments. These tag sequence complements identify the sample fragments from which the sequencing fragments were derived.

DEPR:
A useful modification of this first approach is to prepare template pools from a plurality of different vector libraries as discussed above with reference to FIG. 2A, so that sequencing fragments for a plurality of templates can be generated simultaneously in a single reaction chamber, to reduce the number of template preparations and primer extension reactions. This embodiment is illustrated further in Examples 2 and 3.

DEPR:
In a second approach, sample fragments are inserted into each of a plurality of different tagged vectors (see FIG. 2B, for example), which are then propagated separately, to produce a clonal library for each tagged vector. A clone is selected from at least two of the libraries, and the selected clones are mixed together for subsequent primer extension using a universal primer, size fractionation, and probe hybridization.

DEPR:
When sequencing fragments in the collected aliquots contain primer-tag-primer regions, exponential amplification of the identifier tag sequences can be accomplished by polymerase chain reaction (PCR) using a primer pair that is suitable for amplifying the tag regions. The PCR primer pair is reacted with the target sequencing fragments under hybridization conditions which favor annealing of the primers to complementary regions of opposite strands in the target. The reaction mixture is then thermal-cycled through a selected number of rounds (e.g., 20 to 40) of primer extension, denaturation, and primer/target annealing according to well-known polymerase chain reaction (PCR) methods (e.g., Mullis, 1987, and Saiki, 1985). Linear amplification may similarly be performed for primer-tag-primer regions and tag-primers lacking a second, flanking primer by means of a single extension primer for generating tag-complement sequences. Typically, amplification primers are between 10 to 30 nucleotides in length, and are preferably at least 14 nucleotides long to facilitate specific binding of target, although longer or shorter lengths may also be used.

DEPR:
Typically, amplification primers are pre-loaded in reaction vessels along with the standard nucleotide triphosphates, or analogs thereof, for primer

extension (e.g., ATP, CTP, GTP, and TTP), and any other appropriate reagents, such as MgCl.sub.2 or MnCl.sub.2. A thermally stable DNA polymerase, such as "TAQ", "VENT", or the like, may also be pre-loaded in the reaction vessel, or may be mixed with the sample prior to sample loading. Preferably, amplifications are performed simultaneously on a plurality of collected, same-length sequencing bands, using prefabricated microstructures (e.g., capillary tubes or chips) designed for microscale (small-volume) amplifications. Formats for performing such small-volume amplifications are known and have been described in publications by Wilding et al. (1994), Wittwer et al. (1990, 1991), and Northrop et al. (1993), for example. Preferably, the substrate defining the reaction vessels is formed from silicon or glass, although any other material having high thermal conductivity and which is inert towards amplification reagents may also be used.

DEPR:
A DNA fragment mixture is cloned into a plurality of separate, different tag-vectors (V.sub.k) of the type shown in FIG. 2A, except that universal primer region 56 is omitted, to form a plurality of vector libraries. Each vector includes a cloning site and a first vector primer sequence (P.sub.k) which contains a vector-tag identifier region that is unique for each different-sequence tag-vector V.sub.k. A clone from each library is mixed together to form a template pool in which each different vector clone is uniquely identified by the vector-identifier tag region contained in its vector primer sequence P.sub.k '. The template pool is divided into four aliquots for performing four separate primer extension reactions, one for each terminator base-type. Each of the four aliquots is reacted with a mixture of primer-tag-primers of the form P.sub.u -T.sub.j -P.sub.k to generate sequencing fragments from each different sequence clone simultaneously in the same reaction mixture, where P.sub.u is a universal primer sequence for later PCR amplification of the primer-tag-primer region, T.sub.j is a tag sequence for identifying the terminator base-type and sample fragment, and P.sub.k is a vector-specific primer sequence complementary to each unique vector primer sequence P.sub.k '. For each vector V.sub.k, four different primer-tag-primers of the form P.sub.u -T.sub.j -P.sub.k are used to generate sequencing fragments in each of four separate aliquots, such that for a given vector V.sub.k, four different tags are used (e.g., T.sub.1, T.sub.2, T.sub.3 and T.sub.4), one for each terminator base-type, but P.sub.u and P.sub.k are held constant. Thus, a sequencing fragment mixture generated from first and second template pools each formed from k different vector libraries can be represented as shown in Table 2 below.